

REMARKS

In an Office Action mailed 21 April 2004, claims 31-54, all claims pending in the application, were rejected and the rejection was made FINAL.

Applicants have filed herewith a Request for Continuing Examination together with a Petition for Extension of Time. Applicants' have cancelled all claims previously pending, and submit a new set of claims, claims 56 through 107.

Applicants note with gratitude the Examiner's availability for an interview on 14 July 2004 at the Remsen Building. Applicants' Interview Summary is attached as Appendix 1.

Applicants have attached in an Appendix 2 consisting of translations of the underlying German language priority application, together with published English language abstracts and legal status updates from the EPO.

Applicants' new claims clarify the scope of invention by reciting positively that the identification is one of comparison to a "control." The claims clarify that the method applies to biological polymers generally, especially nucleic acid hybridizations and proteins and Ag-Ab binding. The claimed methods allow one to create a reproduceable high-resolution banding pattern of biopolymers such as chromosomes which banding pattern in turn facilitates the recognition of chromosomal abnormalities by detection of changes in the banding patterns.

In previous Office Actions, Garini et al., U.S. Patent Number 5,817,462 and Cabib et al., U.S. Patent No. 5,784,162, both assigned to Applied Spectral Imaging, had been applied, as well as Mirzabekov et al., U.S. Patent Number 6,458,584.

Garini describes and claims an *in situ* hybridization for which the use of an interferometer system [Claim 1, part b (ii)] is critical to the analysis. Applicants use no such instrument.

Cabib et al., closely related to Garini, also teaches the use of an interferometer.

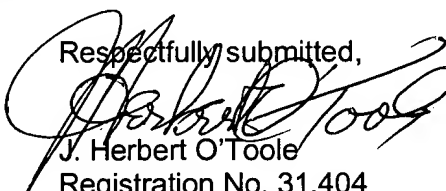
Mirzabekov et al. is directed to micro chip systems which employ fixed probes specific to known mutations indicative of specific characteristics. Applicants use generic probes and obtain information by comparison and allow for the identification of uncharacterized mutations.

Also cited was Shalon et al., Genome Research, 6, 639 (1996), a fluorescent probe method which uses filters to separate wavelengths and does not produce a "false color" as per Applicants' disclosure and claims.

In view of the amended claims and translations provided with this paper, Applicants submit that the application is in condition for Allowance and request examination and expeditious passage to issue.

The Commissioner is authorized to charge applicable fees, or credit any overpayment, to Customer Deposit Account Number 08-0719.

Respectfully submitted,



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Enclosures: Appendix 1, including:
Translation of German Priority Document
English language abstracts (two)
EPO Status update (2 pgs.)
Appendix 2: Interview Summary
Petition for 3- Month Extension of Time (small)
Return Postcard

DE 198 50 661.9

Applicant: MetaSystems Hard & Software GmbH

"Method of Identifying Changes in Biopolymers"

Our Ref.: M 4444 - py / ha

Description

The present invention relates to a method of identifying changes in biopolymers, especially in chromosomal DNA, using two or more different sets of marked or labelled, respectively, detector molecules as well as to a diagnostic kit for detecting these changes.

The representation of human chromosomes has been carried out so far with banding techniques which permit a specific recognition of the chromosomes using light and dark bands (e.g. "G-banding, Q-banding, R-banding"). These banding techniques are based on methods developed by Caspersson et al. (Exp. Cell Res. 60, 1970, 315-319), Sumner et al. (Nature 232, 1971, 31), Seabright et al. (Lancet 2, 1971, 971-972) and Dutrillaux et al. (C R Acad. Sci., Paris, 272, 1971, 3638-3640). However, the identity of individual chromosomal bands can not be defined in every instance with these methods since all bands of all chromosomes appear only either light or dark. This turns out to be a significant disadvantage since chromosomes can be very different morphologically from cell to cell and from tissue to tissue and can possibly comprise translocations (e.g. in the case of tumors) the recognition of which can be of particular significance for the person to be examined. This applies, e.g. to the realization of the desire for children in the case of balanced translocations ("exchanges of parts of chromosomes") of one parent, to the recognition of the cause of malformations in children with and without mental retardation and to the diagnosis of leukemias and other tumors which frequently exhibit specific chromosomal changes with diagnostic and therapeutic significance.

Fluorescence in-situ hybridization (FISH) was described for the first time for the routine in practicable form by Pinkel et al. (Proc. Natl. Acad. Sci. USA 83, 1986, 2934-2938) as a suggestion for solving this problem. Today, all human

chromosomes of a metaphase can be represented in different colors with this method by using chromosome-specific DNA libraries (chromosome painting, 24-color FISH, Schröck et al., Science 273, 1996, 496-497; Speicher et al., Nature Genet. 12, 1996, 368-375) and by the use of vectors, e.g. cosmids, pacs or YACs, which can contain different amounts of human DNA, specific chromosomal regions can be re-checked in their integrity via multicolor techniques by means of FISH. Also parts of genes and repetitive DNA elements can be identified in this way regarding their chromosomal localization and their presence or absence, respectively. However, a multicolor representation of chromosomal sections at the band level has not been possible so far.

Thus, the problem underlying the present invention is to provide a novel and improved method for the identification of, in particular, changes in chromosomal DNA which method should make possible a multicolor representation at the band level.

This problem is solved by the embodiments of the present invention characterized in the claims.

In particular, a method of identifying changes in biopolymers as target molecules using two or more different sets of marked detector molecules is provided in which at least two sets are specific for a certain region in the target molecules and the markings or labellings of the particular detector molecules of these sets specific for a certain region in the target molecules are different, comprising the steps of

- (a) carrying out binding reactions between the detector molecules of the different sets and between the target molecules, wherein the particular marked detector molecules of at least two sets bind in such a manner to a certain region of the target molecules that the different markings of the detector molecules overlap, and
- (b) qualitative and quantitative evaluation of the bonds obtained in this manner via the different markings of the detector molecules.

The term "biopolymers as target molecules" means DNA, preferably chromosomal DNA, RNA or polypeptides. The target molecules may be appropriately arranged or immobilized, respectively, prior to carrying out the method of the present invention, in particular prior to step (a), e.g. by gel electrophoretic separation in a suitable matrix or by fixing or arranging, respectively, e.g. metaphase chromosomes or interphase nuclei on a suitable carrier.

The term "marked detector molecules" means nucleic acids or antibodies having at least one marking. The antibodies may be present polyclonally or monoclonally. The terms "nucleic acid" and "nucleic-acid sequence" and "nucleic-acid probes", respectively, mean native, semisynthetical or modified nucleic-acid molecules of deoxyribonucleotides and/or ribonucleotides and/or modified nucleotides such as amino nucleotides or [α -S]-triphosphate nucleotides. In a preferred embodiment of the present invention the nucleic acids stem from chromosomal DNA from, e.g. mammals such as homo sapiens sapiens. The chromosomal DNA as detector molecules is present in vectors, e.g. cosmids or YACs or stems from chromosomal or chromosome region-specific DNA libraries which can be obtained, e.g. via microdissection methods or laser-activated flow-cytometric sorting of specific chromosomes and, if required, subsequent amplification by, e.g. DOP-PCR.

The term "marking" means suitable, directly or indirectly detectable atoms or molecules which are introduced into the detector molecules or connected to them. Suitable markings are, e.g. those comprising fluorescent dyes coupled to nucleotides and/or those comprising, e.g. biotin and/or digoxigenin and/or nucleotides marked with radioactive isotopes. In a preferred embodiment the marker compound is a fluorescent dye having a difference, sufficient for the selection of small amounts of substance, in the fluorescence behavior of the emission spectra such as, e.g. coumarins and rodamins and/or in the fluorescence life time such as, e.g. fluorescent isothiocyanates and europium-chelate-marked and/or porphyrin-marked avidines.

The term "binding reaction" means a hybridization, preferably an *in-situ* hybridization, or an antigen/antibody reaction dependent on the selection of the detector molecules

and/or of the target molecules. The term "*in-situ* hybridization" means the apposition of a synthetically produced DNA/RNA probe provided with biological, physical or chemical markings for detection as detector molecule to native DNA/RNA sequences occurring in nature, wherein the apposition is achieved by denaturing and renaturing of the appropriate nucleic acids. Of course, these DNA/RNA probes contain at least one sequence section capable of hybridizing with a DNA/RNA sequence of the target molecule, such as a chromosome. This sequence section comprises a specific, individually present sequence region of the detector molecule which region is preferably 100 to 1,000 base pairs long and which attaches to a complementary region of the target molecule under formation of hydrogen bridges at a suitable temperature, preferably at 50°C or less and at a suitable salt concentration containing preferably 50-300 mmol/l monovalent ions and 0-10 mmol/l bivalent ions. The binding reaction of the particular sets of marked detector molecules may be carried out simultaneously or successively.

The expression "set of detector molecules" means detector molecules which are specific for a certain region of the target molecules. This set of detector molecules may be, e.g. chromosomal DNA present in vectors or may be a chromosome-specific DNA library. The markings of the detector molecules in the set may be the same or different, e.g. containing three different markings.

The expression "at least two or more different sets of marked detector molecules" means the presence of at least one pair of different sets, wherein the sets of this pair bind in a certain area or region, respectively, of the target molecules in such a manner that at least the different markings of the particular detector molecules, preferably the binding sites of the particular detector molecules of these different sets, overlap. This property, according to the invention, of a pair of different sets means that the particular detector molecules in the different sets of a pair which are produced or obtained, respectively, in an overlapping manner from this certain region of the target molecules can be used as a standard or for comparative examination with appropriately processed specimens from patients, respectively. In an embodiment according to the invention the detector molecules of a set are preferably

designed in such a manner that after the hybridization the detector molecules are bound in a continuously changed concentration, preferably in the manner of a Gauss distribution, in the longitudinal direction to the target molecules, e.g. chromosomes.

The qualitative and quantitative evaluation of the bonds obtained in step (a) via the different markings of the detector molecules, which evaluation is characterized in step (b) of the method according to the invention, may be used, by employing a scanning device or a device, respectively, for directed scanning, e.g. along or in the longitudinal direction of, respectively, the chromosome to be investigated. Such a scanning device is, e.g. a fluorescence microscope. Image-generating signals can be taken by the scanning device via an image processing unit, e.g. a CCD camera, via the physical and/or chemical and/or biological markings of the detector molecules which have been attached to the desired target molecules which image processing unit processes the individual signals of the different markings in a suitable manner supported by a computer. The intensities or the intensity ratios, respectively, of the different markings in the regions of overlapping and non-overlapping markings of the particular detector molecules can be recorded and evaluated qualitatively and quantitatively, preferably in the longitudinal direction of the target molecules, particularly of fixed metaphase chromosomes, with this image processing unit coupled to the scanning device.

Further subject matter of the present invention is constituted by a diagnostic kit for the identification of changes in above-defined biopolymers as target molecules containing at least two different sets of marked detector molecules in accordance with previous definitions.

In particular, the kit according to the invention can be used for the identification or exclusion, respectively, of chromosomal modifications or aberrations, respectively, in human genetics such as balanced chromosome rearrangements, which are, as is known, of great significance for the realization of the desire for children in the case of carriers of such a change, balanced and unbalanced chromosome changes as the cause of malformations and/or mental retardation and in the tumor diagnosis of solid

tumors as well as of hematological neoplasias (AML, ALL, MDS, and others) on the one hand for the detection of known alterations relevant to prognosis and on the other hand for the determination of further, previously unknown alterations.

Further subject matter of the present invention relates to an automatic correction by adding a localized DNA probe.

A monochrome CCD camera in combination with specific fluorescence filters is used when recording chromosome region-specific specimens marked with different fluorochromes like the specimens used for the method of multicolor banding. The signals of the individual fluorochromes are recorded successively as individual images and subsequently combined to a color image. A shift of the position of the individual images relative to each other on account of optical influences of the filters (different wedge errors, parallel shift due to slight tilting in the path of the rays) can not be excluded thereby. An interactive or automatic correction, e.g. by a correlation of the individual images, is not possible with the required precision since the at most partially overlapping probes do not have any common structures which can be used for a subsequent superpositioning. Every slight shift results in the evaluation of the intensity ratios in artifacts in the banding pattern.

An automatic correction is made possible by adding a localized DNA probe which is marked at the same time with all fluorochromes used in the method according to the invention. A structure which is identical in all individual images is available therewith for the automatic correction of position. The correction of position may take place, e.g. via a determination of the center of intensity of the probe in each individual image and by a subsequent relative shifting of the individual images in such a manner that the centers of the individual images come to be located at the same position.

Also distortions of the images relative to each other can be determined and corrected by the use of two different probes.

The use of even more probes basically makes possible the correction of more complex transformations of position than translation and rotation such as, e.g. changes of scale.

Furthermore, it can be advantageous in a further preferred embodiment of the present invention to add calibrating probes (DNA probes or fluorescent particles) of known intensity which can serve for standardizing the intensities of the fluorescent signals to be evaluated.

Furthermore, it can be advantageous in a further preferred embodiment of the present invention to add DNA probes whose exact localization within the genome is known and which can be used for establishing the relation between color bands and the ISCN bands.

The figures show:

Fig. 1 is a photographic representation for the qualitative and quantitative evaluation of the localization of region-specific colorations in chromosome 5. In the upper part of this figure the distribution of the marked detector molecules in the longitudinal direction of the chromosome as well as intensities of the different markings of the detector molecules are shown graphically.

Fig. 2 is a tabular presentation of the marking pattern of the region-specific chromosome section of chromosome 5 shown in Fig. 1. Cy5, TR (Texas Red), Cy5.5, SO (Spectrum Orange), SG (Spectrum Green) are the different fluorescent dyes which were used to mark the individual region-specific DNA libraries. The assignment is characterized by a solid square: (■). The markings resulting from the overlapping of the DNA libraries in the corresponding regions are rendered recognizable by an empty square (□).

Fig. 3 shows the respective homologous normal chromosomes 5 from two different metaphase plates with multicolored banding. The presentation makes it clear

that the banding pattern is identical on the homologous chromosomes and can even be reproduced from metaphase plate to metaphase plate.

Fig. 4 shows a photographic representation of a multicolored FISH of a metaphase plate with complex chromosomal modifications.

Fig. 5 shows chromosomes 5 in a case with acute myeloid leukemia. The respective normal chromosome 5 is shown on the right side and the chromosome on the left side displays an interstitial deletion in the long arm.

The following example illustrates the invention.

Example

A total of 7 overlapping chromosome region-specific microdissection libraries were produced for the multicolor band pattern of chromosome 5 (Meltzer et al., *Nature Genet.* 1, 1992, 24-28). The p-arm of chromosome 5 was subdivided for this into two regions, the q-arm into four regions. 8-10 fragments per chromosome region were isolated with a finely pulled-out glass needle from the microscope slide under microscopic view (Senger et al., *Hum. Genet.* 84, 1990, 507-511). The thus obtained DNA was amplified via a DOP-PCR (degenerate oligonucleotide polymerase chain reaction, Telenius et al., *Genomics* 13, 1992, 718-725; Zhang et al., *Blood* 81, 1993, 3365-3371). In a subsequent reaction these chromosome region-specific DNA libraries were partially marked directly with fluorochromes present coupled to nucleotides (e.g. Spectrum Orange-dUTP, Spectrum Green-dUTP, both Vysis, and Texas Red-dUTP, Molecular Probe). In another part, DNA libraries were marked with nucleotides coupled to haptenes (e.g. biotin-dUTP and digoxigenin-dUTP, Boehringer, Mannheim). After the hybridization has taken place haptenes can be detected with suitable detection reagents (e.g. avidine-Cy5, Amersham, and anti-digoxigenin IgG, Boehringer, Mannheim, which is coupled to Cy5.5, Mab labeling kit, Amersham).

The hybridization, washing steps and detection are carried out according to standard protocols (Senger et al., Cytogenet. Cell Genet. 64, 1993, 49-53).

The analysis is carried out, e.g. with a fluorescence microscope equipped with suitable filter sets. Separate images are taken for each color channel, which images can be subsequently processed further with a computer.

A characteristic feature of the partial "painting" probes obtained by microdissection is a continuously weakening fluorescent signal in the border regions. By simultaneous overlapping of the probes and, therefore, of the fluorescent signals of two adjacent partial "painting" probes brings about a continuously changing ratio of the fluorescence intensities along chromosome 5. If a chromosome stained in this manner is subdivided into several (20-25) small sections, a false color stain can be assigned to each of these sections via a suitable computer program on the basis of the relative fluorescence intensities of all fluorochromes used. This assignment gives rise to a colored band pattern along a chromosome, in this case chromosome 5. The same combination of fluorescence relationships and false colors can be used for all further hybridizations with the same specimen set.

Since the hybridization behaves in a sufficiently constant manner the band pattern is also correspondingly reproducible (figure 3). A loss of the resolving power in the case of shorter chromosomes, as is known from previously customary banding methods (e.g. GTG banding) is not observed in this case. A reproducible pattern of at least 25 bands is achieved for chromosome 5. This corresponds to a band level of approximately 550 bands per haploid chromosome set.

It is possible with the aid of this method to identify changes in chromosomes independently of their condensation state. This is particularly significant in tumor cytogenetics, too. Tumor chromosomes often display a low resolution of the band pattern, which makes the recognition of chromosomal changes significantly more difficult. It is therefore to be assumed that previously unknown cytogenetic changes are present in tumors which possibly represent an important prognosis factor and

could therefore be of significance, e.g. for a risk-adapted therapy. According to the invention at least 25 bands can be achieved even on tumor chromosomes after hybridization with the specimen set for chromosome 5 described in detail above (figure 5).

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Applicant: MetaSystems Hard & Software GmbH

"Method of Identifying Changes in Biopolymers"

Our Ref.: M 4444 - py / ha

Claims

1. A method of identifying changes in biopolymers as target molecules using two or more different sets of marked detector molecules in which at least two sets are specific for a certain region in the target molecules and the markings of the particular detector molecules of said sets specific for a certain region in the target molecules are different, comprising the steps of
 - (a) carrying out binding reactions between the detector molecules of the different sets and between the target molecules, wherein the particular marked detector molecules of at least two sets bind in such a manner to a certain region of the target molecules that the different markings of the particular detector molecules overlap, and
 - (b) qualitative and quantitative evaluation of the bonds obtained in this manner via the different markings of the detector molecules.
2. The method according to claim 1, wherein the target molecules are immobilized before step (a).
3. The method according to claim 2, wherein the target molecules or the target molecule are (is) arranged on a carrier or in a matrix.
4. The method according to anyone of claims 1 to 3, wherein the binding reactions of the particular sets of marked detector molecules are carried out simultaneously or successively.

5. The method according to anyone of claims 1 to 4, wherein the binding reaction in step (a) is a hybridization or an antigen/antibody reaction.
6. The method according to claim 5, wherein the hybridization is an *in-situ* hybridization.
7. The method according to anyone of claims 1 to 6, wherein the target molecules are nucleic acids or polypeptides.
8. The method according to claim 7, wherein the nucleic acids are selected from DNA and RNA.
9. The method according to claim 7 or 8, wherein the nucleic acids are chromosomal DNA.
10. The method according to anyone of claims 1 to 9, wherein the marked detector molecules are selected from nucleic acids or antibodies.
11. The method according to claim 10, wherein the particular sets of nucleic acids stem from different chromosome-region-specific DNA libraries.
12. The method according to claim 10 or 11, wherein each set of detector molecules contains one or more different markings.
13. The method according to claim 12, wherein the marking comprises a fluorescent dye.
14. The method according to anyone of claims 1 to 13, wherein the evaluation in step (b) is carried out using a scanning device with which the intensities or intensity ratios, respectively, of the markings in the regions of overlapping and non-overlapping markings of the particular detector molecules are recorded in the longitudinal direction of the target molecules.

15. A diagnostic kit for identifying changes in biopolymers as target molecules, containing at least two different sets of marked detector molecules according to definition in anyone of the claims.
16. The use of the kit according to claim 15 for identifying chromosomal modifications in human genetics and in tumor diagnostics.
17. The method according to anyone of claims 1 to 14, wherein at least one DNA probe marked with at least two of the N-fluorochromes used is added for determining an optically caused, relative shift of the image information of the fluorochromes and for their positional correction.
18. The method according to claim 17, wherein at least one DNA probe marked with all N used fluorochromes is added for the simultaneous positional correction of all fluorochromes.
19. The method according to claim 17, wherein N-1 DNA probes are marked with two suitable fluorochromes each and the positional correction is carried out pairwise.
20. The method according to anyone of claims 17 to 19, wherein positional transformations with more degrees of freedom are corrected by using a sufficient number of DNA probes.
21. The method according to anyone of claims 17 to 20, wherein the determination of the relative shifts and the positional correction take place interactively.
22. The method according to anyone of claims 17 to 21, wherein the determination of the relative shifts and the positional correction take place automatically.

23. The method according to anyone of claims 17 to 22, wherein calibrating probes of a known or reproducible, respectively, constant intensity are added for standardizing the signal intensities.
24. The method according to claim 23, wherein the calibrating probes are fluorescence-marked DNA probes.
25. The method according to claim 23, wherein the calibrating probes are fluorescence-marked particles.
26. The method according to anyone of claims 17 to 25, wherein the calibrating probes are simultaneously used for positional correction.
27. The diagnostic kit according to claim 15, which additionally contains suitable probes for positional correction.
28. The diagnostic kit according to claim 27, which additionally contains suitable calibrating probes for intensity standardization.
29. The diagnostic kit according to claim 27 or 28, wherein additional DNA probes are added for a direct assignment of color bands to the ISCN nomenclature.
30. The diagnostic kit according to anyone of claims 27 to 29, wherein said probes are simultaneously used for positional correction and/or intensity standardization.

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Applicant: MetaSystems Hard & Software GmbH

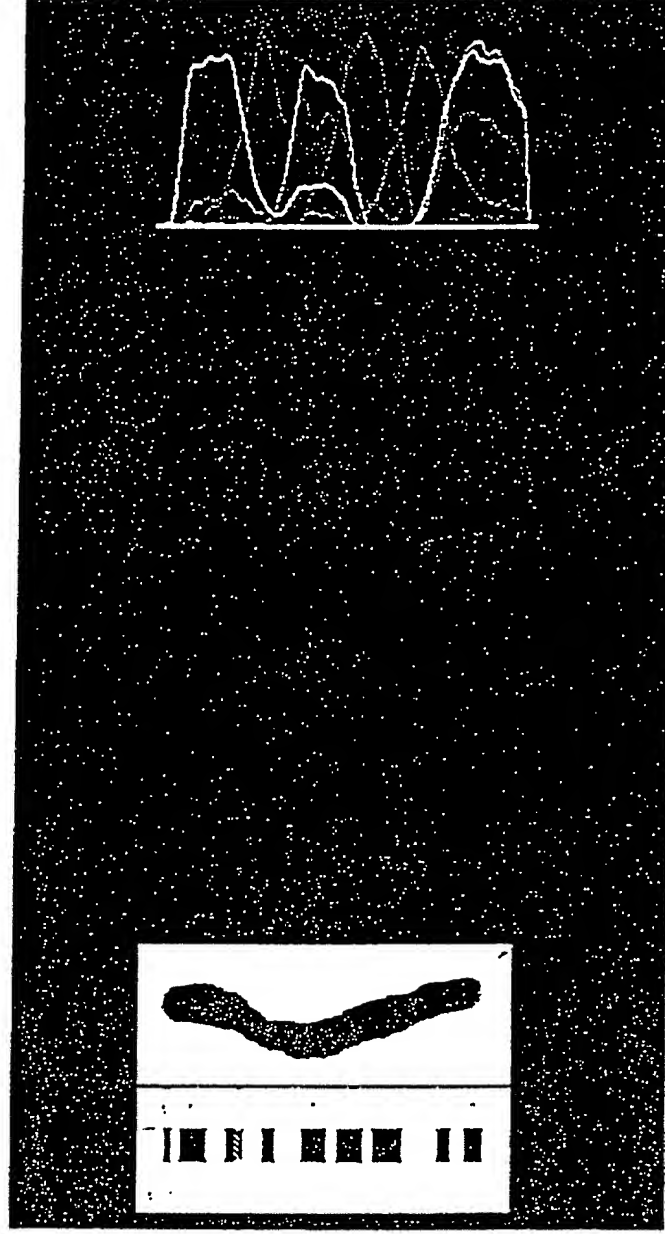
"Method of Identifying Changes in Biopolymers"

Our Ref.: M 4444 - py / ha

Abstract

The present invention relates to a method of identifying changes in biopolymers, especially in chromosomal DNA, using two or more different sets of marked or labelled, respectively, detector molecules as well as to a diagnostic kit for detecting these changes.

Fig. 1



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Fig. 2

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region-specific DNA library	Cy 5	TR	Cy 5.5	SO	SG
1	■				
1/2	□	□			
2		■			
2/3		□	□		
3			■		
3/4			□	□	
4				■	
4/5				□	□
5					■
5/6	□		□		□
6	■		■		

10

Fig. 4

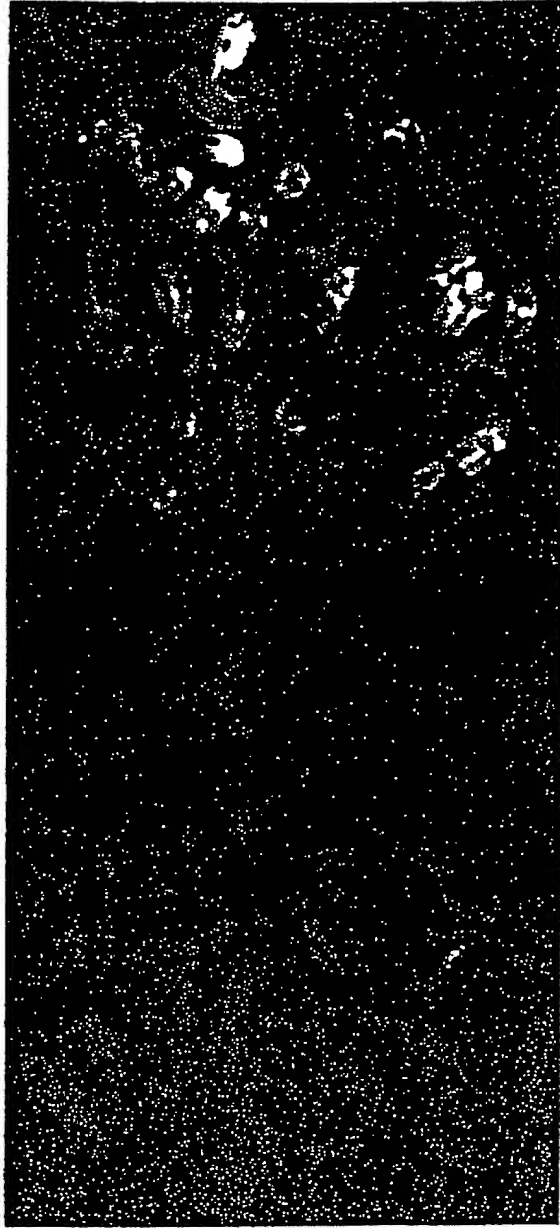


Fig. 3



Fig. 5



Method for the detection of modifications in biopolymers

Patent number: EP0957176
Publication date: 1999-11-17
Inventor: PLESCH ANDREAS DR (DE); CHUDoba ILSE DR (DE); LOERCH THOMAS DR (DE); SENGER GABRIELE DR (DE)
Applicant: METASYSTEMS HARD & SOFTWARE GM (DE)
Classification:
- international: C12Q1/68; G02B21/00
- european: G01N33/53F, G01N33/574, C12Q1/68B14
Application number: EP19990101714 19990210
Priority number(s): DE19981006303 19980216; DE19981050661 19981103

Also published as:

J P2000201699 (A)

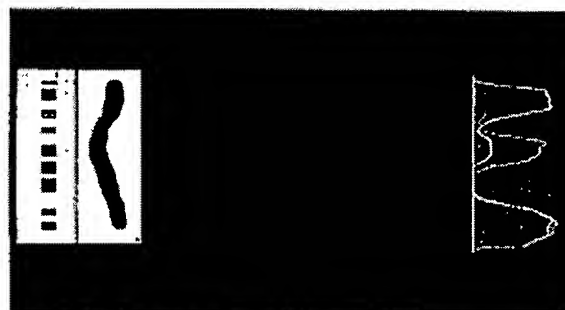
Cited documents:

W O9740191

Abstract of EP0957176

Detecting change in bio-polymers using at least two different sets of detector molecules which are specific for one site of the bio-polymer is new, comprising reacting target molecules with the detector molecules which react with the target molecules and their different labels are detected. The method quantitatively and qualitatively determining the changes in the bio-polymer. An Independent claim is also included for a diagnostic kit for carrying out the method above.

fig. 1





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IDENTIFICATION OF MUTATION IN BIOPOLYMERS

Patent number: JP2000201699
Publication date: 2000-07-25
Inventor: CHUDoba ILSE; LOERCH THOMAS DR; PLESCH ANDREAS DR
Applicant: METASYSTEMS HARD & SOFTWARE GMBH
Classification:
- international: C12Q1/68; G01N33/53; G01N33/566
- european:
Application number: JP19990037248 19990216
Priority number(s):

Also published as:

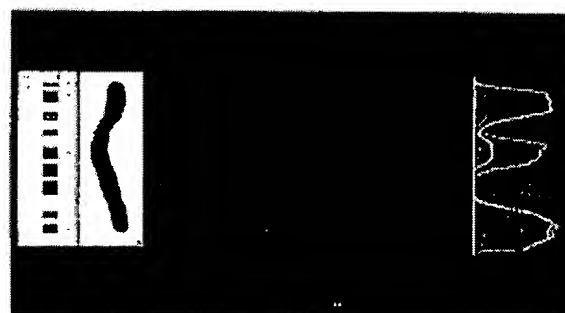
 E P0957176 (A1)
 J P2000201699 (A)

Abstract of JP2000201699

PROBLEM TO BE SOLVED: To identify the mutation of biopolymers by reaction the biopolymer of the target molecule with plural sets of detection molecules that have different labels from each other and are specific in the areas in which the target molecule distributes and evaluating the bonds via the labels different in their detecting molecules.

SOLUTION: The mutations in a biopolymer as a target molecule are identified by using labeled detection molecules of different two or more sets. In this case, at least two sets of specifically labeled detection molecules react between different sets of the detection molecules and between the target molecules so that the labels different detection molecules may be duplicated and the bonds obtained through the different labels are evaluated qualitatively and quantitatively, thereby the mutation in the biopolymer, for example, chromosomal DNA or the like can be identified.

fig. 1



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17.11.1999 Patentblatt 1999/46

(51) Int. Cl.⁶: **C12Q 1/68, G02B 21/00**

(21) Anmeldenummer: 99101714.6

(22) Anmeldetag: 10.02.1999

(84) Benannte Vertragsstaaten:
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MC NL PT SE**
Benannte Erstreckungsstaaten:
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(30) Priorität: 16.02.1998 DE 19806303
03.11.1998 DE 19850661

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(54) **Verfahren zum Nachweis von Änderungen in Biopolymeren**

(57) Die vorliegende Erfindung betrifft ein Verfahren zum Nachweis von Änderungen in Biopolymeren, insbesondere in chromosomaler DNA, unter Verwendung von zwei oder mehreren unterschiedlichen Sätzen von

markierten Detektormolekülen sowie einen diagnostischen Kit zum Nachweis dieser Änderungen.

Fig. 1



Method for the detection of modifications in biopolymersLegal status (INPADOC) of **EP0957176**

EP F	99101714 A	(Patent of invention)
PRS Date :	1999/11/17	
PRS Code :	AK	
Code Expl.:	+ DESIGNATED CONTRACTING STATES:	
KD OF CORRESP. PAT.:	A1	
DESIGNATED COUNTR.:	AT BE CH DE DK ES FI FR GB GR IE IT LI NL PT SE	
PRS Date :	1999/11/17	
PRS Code :	AX	
Code Expl.:	+ EXTENSION OF THE EUROPEAN PATENT TO	
FURTHER INFORMATION:	AL;LT;LV;MK;RO;SI	
PRS Date :	2000/04/19	
PRS Code :	17P	
Code Expl.:	+ REQUEST FOR EXAMINATION FILED	
EFFECTIVE DATE:	20000217	
PRS Date :	2000/07/26	
PRS Code :	AKX	
Code Expl.:	+ PAYMENT OF DESIGNATION FEES	
FURTHER INFORMATION:	AT BE CH DE DK ES FI FR GB GR IE IT LI NL PT SE	
PRS Date :	2002/04/17	
PRS Code :	17Q	
Code Expl.:	+ FIRST EXAMINATION REPORT	
EFFECTIVE DATE:	20020305	

INTERVIEW SUMMARY

July 14, 2004

United States Patent and Trademark Office

U.S. Serial Number: 09/250,466
Inventors: Chudoba et al.
Filed: 16 February 1999
Entitled: METHOD OF IDENTIFYING CHANGES IN BIOPOLYMERS
Attorney Docket: 25048-015

An interview was held at the Remsen Building, 2nd floor of the United States Patent and Trademark Office on the Carlyle Campus.

For USPTO: Marjorie A. Moran (Primary Examiner, GAU 1631)

For Applicant: John B. Hardaway, III, Reg. No. 26,554

J. Herbert O'Toole, Reg. No. 31,404

A set of proposed claims (attached) were discussed.

It was agreed that the recitation of "filters" in part c of Claim 101 removes Garini, U.S. Patent Number 5,817,462 as a §102 reference.

Literal support for term "pseudo value" appeared to be lacking in the specification. "False value" has support. Literal support for "identity" of a biopolymer appears to be lacking. "Comparing" or "comparison" was suggested as an alternative.

Proposed claim 104 is unclear as to whether "microdissected" limits the biopolymer or the method.

Filters as part of the method to distinguish over Garini needs literal support in the specification.

The Cabib reference, U.S. Patent Number 5,784,162 is cited for use of filters in optical spectrometric analysis of tissues. Miczabekov, U, S. Patent Number 6,458,584 was distinguished on the basis of the employment of specific probes to identify abnormal sequences.

Attachment: Proposed Claims

Interview Summary	Application No. 09/250,466	Applicant(s) CHUDOBA ET AL.	
	Examiner Marjorie A. Moran	Art Unit 1631	

All participants (applicant, applicant's representative, PTO personnel):

(1) Marjorie A. Moran.

(3) J. Hardaway

(2) H.O. Toole

(4) _____

Date of Interview: 7/14/04

Type: a) ☐ Telephonic b) ☐ Video Conference
c) ☐ Personal [copy given to: 1) ☐ applicant 2) ☒ applicant's representative]

Exhibit shown or demonstration conducted: d) ☐ Yes e) ☐ No.
If Yes, brief description: _____

Claim(s) discussed: Proposed claims and clms 47-49

Identification of prior art discussed: GARNI, CABIB

Agreement with respect to the claims f) ☐ was reached. g) ☐ was not reached. h) ☒ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: see below

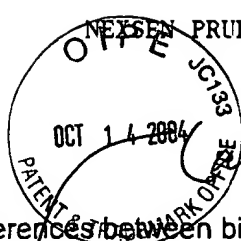
(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

The examiner pointed to possible problems with support for proposed claim limitations, but agreed that the 102 rejections of record would not apply to the proposed claims. No agreement on possible obviousness (103-type) rejections was reached. Other limitations which may be free of the prior art were discussed, but no consensus was reached.

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

MA Moran
Examiner's signature, if required



101. A method for identifying differences between biopolymers in situ comprising:

- a) isolating biopolymers on a substrate;
- b) reacting said isolated biopolymers with at least two different sets of labeled detector molecules, each set being a specific binding partner for a portion of said isolated biopolymers;
- c) recording the intensity, and the intensity ratios, of the labels along the length of said isolated biopolymer using filters to differentiate labels;
- d) analyzing the recorded data to determine the identity of the biopolymer monomers in areas where the labeled detector molecules overlap, and assigning a pseudo value to the location of overlap; and
- e) comparing the result to that obtained using a different biopolymer.

102. A method according to claim 101 wherein the biopolymer is a sequence of nucleic acids.

103. A method according to claim 101 wherein the biopolymer is a polypeptide.

104. A method according to claim 101 wherein said biopolymer has been micro dissected. ✓

105. A method according to claim 102 wherein the labeled detector molecules are labeled nucleic acid probes.

106. A method according to claim 105 wherein the labeled detector probes are fluorescent

~~prints~~ dyes
pseudo = deletion
" = repeat

101. A method for identifying differences between biopolymers in situ comprising:

- a) isolating biopolymers on a substrate;
- b) reacting said isolated biopolymers with at least two different sets of labeled detector molecules, each set being a specific binding partner for a portion of said isolated biopolymers;
- c) recording the intensity, and the intensity ratios, of the labels along the length of said isolated biopolymer using filters to differentiate labels;
- d) analyzing the recorded data to determine the identity of the biopolymer monomers in areas where the labeled detector molecules overlap, and assigning a pseudo value to the location of overlap; and
- e) comparing the result to that obtained using a different biopolymer.

102. A method according to claim 101 wherein the biopolymer is a sequence of nucleic acids.

103. A method according to claim 101 wherein the biopolymer is a polypeptide.

104. A method according to claim 101 wherein said biopolymer has been micro dissected.

105. A method according to claim 102 wherein the labeled detector molecules are labeled nucleic acid probes.

106. A method according to claim 105 wherein the labeled detector probes are fluorescent paints.

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Dr. Ralf Perrey Muller Bore & Partner	011 49 89 45 06 7450	

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PHONE: 864-282-1185

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